Supplementary Information

1. Supplementary Methods Summary

1.1 Promoter Analysis of the *TYK2* Gene

The PCR-amplified *TYK2* promoter fragment, with either wild or variant type sequences, was cloned into a pGL4.17[luc2/Neo] vector (Promega). Insert was prepared from wild type *TYK2* or homozygous *TYK2* promoter variant by PCR amplification using forward primer 5'-AAAGCTAGCAGCTGCCCTGTGAGGAGGC-3' and reverse primer 5'-AAAAAGCTTTCCCCGCGGCTTCTTCCTGA-3', which led to a 1572bp product. Luciferase assay was conducted by transfection of vectors to 293T cells with 24-well plates. Luciferase activity was measured 24 hours after transfection in 293T cells using a dual luciferase assay kit (Promega Corporation, Madison, WI). The experiments were repeated five times.

1.2 Expression of *TYK2* gene and interferon stimulated genes (ISGs), including PKR, OSR and MxA gene

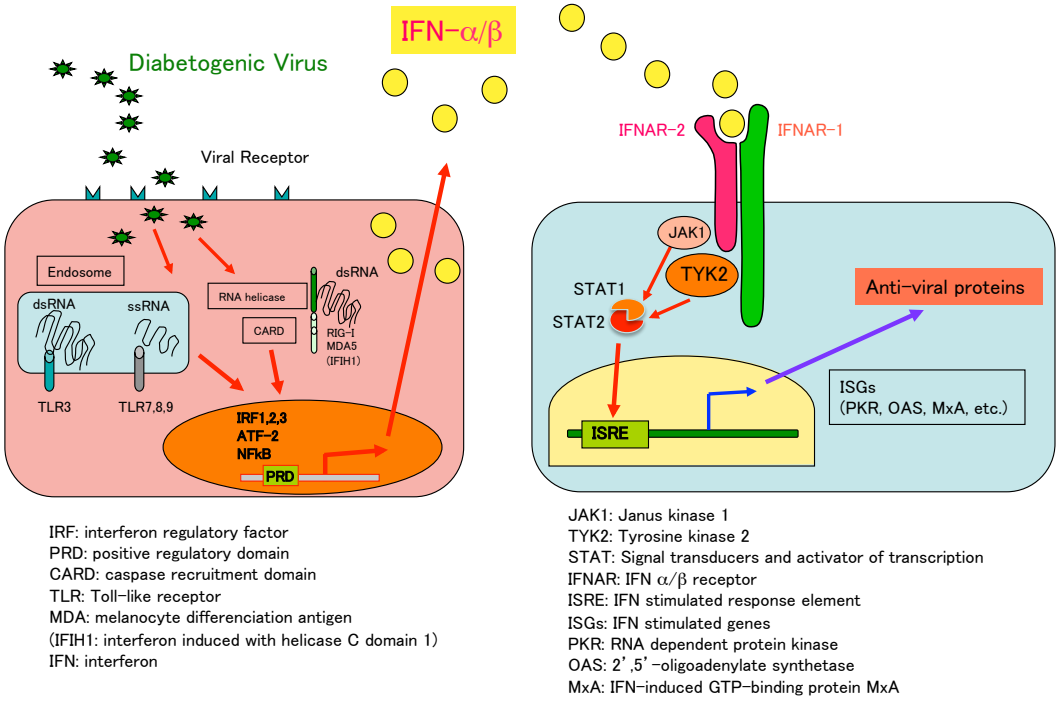
Patients with type 2 diabetes, possessing either *TYK2* wild type or promoter variant, were studied for the expression of *TYK2* gene, *JAK1* gene and interferon-stimulated genes before and after IFN-β stimulation. 14 patients with type 2 diabetes (age, 65.1±10.8; HbA1c, 7.3±0.8%) carrying heterozygous (n=12) and homozygous (n=2) *TYK2* promoter variant, and 17 patients with type 2 diabetes (age, 71.8±9.9; HbA1c, 7.1±0.6%) carrying wild type *TYK2* promoter were studied. The data are expressed as means±standard deviations.

PBMCs were isolated by LSM (MP BIOMEDICALS, Ohio, USA) from patients. PBMCs were stimulated with IFN-β (500U/ml) (SIGMA-ALDRICH, Missouri, USA) for 12h, after which total RNA was extracted using ISOGEN (Wako Chem., Tokyo). cDNA was synthesized from the RNA template (1 μg) with High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems) according to the manufacturer protocol. Quantitative PCR was carried out by using an ABI 7500 real-time PCR system with Power SYBER green Master Mix (Applied Biosystems). The PCR was set up under the following thermal cycling conditions: 50℃ 2min, 95℃ 10min, followed by 40 cycles of 95℃ 15sec, and 63℃ 1 min. Fluorescence signals were collected by the machine using the extension phase of each PCR cycle. The threshold cycle value was normalized to that of β-actin. The qPCR was performed by using the following primer pairs: for human *TYK2* gene, 5'-TGGCATGAATCCTCGGGAAC-3' and 5'-CATGCTTGCCCTGCTCAAAG-3'; *JAK1* gene, 5'-CTACAGTCTGCACGGTTCGGA-3' and 5'-CGATCGAAACTCAGTTGGCTC-3'; Protein kinase R (PKR) gene, 5'-TCTGACTACCTGTCCTCTGGTTCT-3' and 5'-GCGAGTGTGCTGGTCACTAAAG-3'; 2'-5' oligoadenylate synthetase (OAS) gene, 5'-ACCTGGTTGTCTTCCTCAGTCC-3' and 5'-GAGCCTGGACCTCAAACTTCAC-3'; myxovirus resistance A (MxA) gene, 5'-TTCGGCTGTTTACCAGACTCC-3' and 5'-CAAAGCCTGGCAGCTCTCTAC-3'; β-actin gene, 5'-GCACCACACCTTCTACAATGAGC-3' and 5'-GGATAGCACAGCCTGGATAGCAAC-3'.

The experiments were repeated three times. The relative mRNA level was expressed as fold change relative to the value of the corresponding healthy non-diabetic control. Statistical analysis was done by Student's t-test.

2. Supplementary Figures

Supplementary Figure 1.



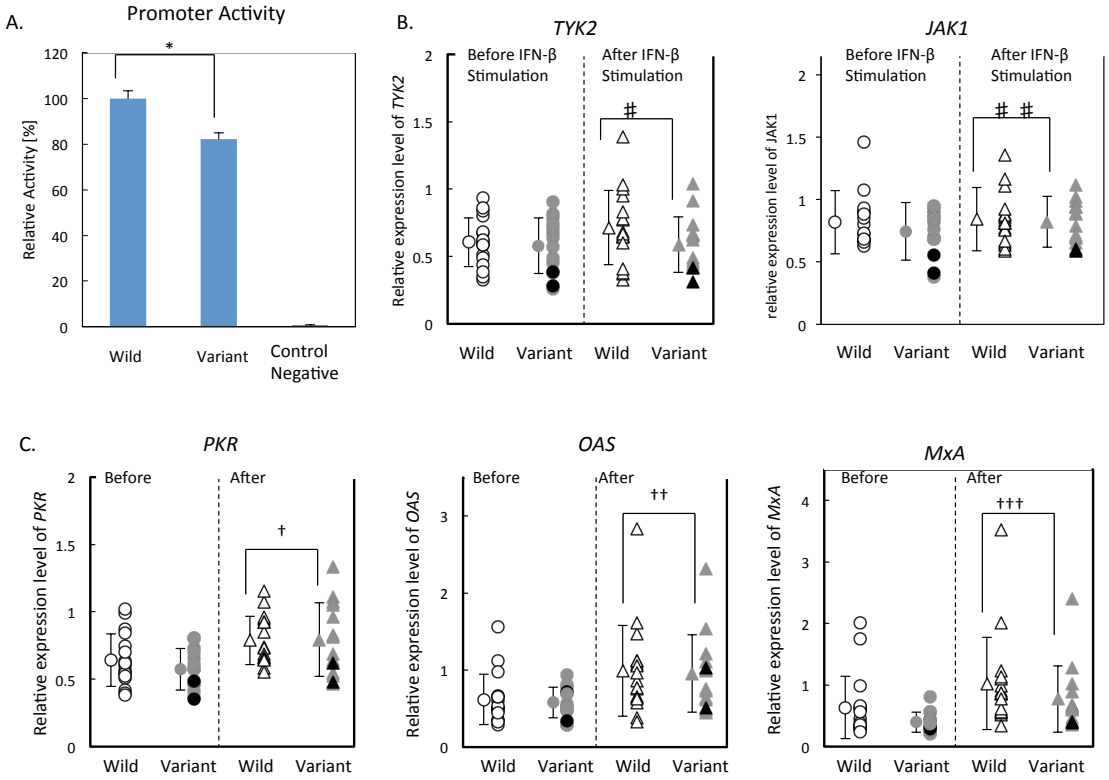
IRF: interferon regulatory factor
PRD: positive regulatory domain
CARD: caspase recruitment domain
TLR: Toll-like receptor
MDA: melanocyte differenciation antigen
(IFIH1: interferon induced with helicase C domain 1)
IFN: interferon

JAK1: Janus kinase 1
TYK2: Tyrosine kinase 2
STAT: Signal transducers and activator of transcription
IFNAR: IFN α/β receptor
ISRE: IFN stimulated response element
ISGs: IFN stimulated genes
PKR: RNA dependent protein kinase
OAS: 2',5'-oligoadenylate synthetase
MxA: IFN-induced GTP-binding protein MxA

Supplementary Figure 1. Type 1 interferon (IFN-α/β) production in response to putative diabetogenic virus infection and IFN signaling pathway.

JAK1 and TYK2 are reciprocal IFN receptor-associated molecules, mediating the downstream signal to induce IFN-stimulated genes (ISGs) to resist against viral infection. (modified from Diabetes and Viruses 2013, Springer Science-Media, p41, Fig.5.3)

Supplementary Fig. 2.



Supplementary Figure 2. Promoter assay of *TYK2* promoter variant and expression level of *TYK2*, *JAK1*, and interferon-stimulated genes.

Diabetic patients with heterozygous *TYK2* promoter variant type were compared with those with wild type *TYK2* gene. A. Promoter activity of *TYK2* promoter variant was assessed by the luciferase assay. Relative activity of the luciferase assay of *TYK2* promoter activity was expressed as percent, compared with that of wild type (100%). Mutated *TYK2* promoter variant showed significantly reduced promoter activity (82.6%±0.21) (n=9). (*P<0.001) B. Relative expression level of *TYK2* gene induced by interferon-β (IFN-β) stimulation in patients with diabetes possessing heterozygous *TYK2* promoter variant (n=12) (●: before stimulation, ▲: after IFN-β stimulation) and homozygous patients (n=2) (●: before stimulation, ▲: after IFN-β stimulation) (before stimulation, 0.58±0.21; after stimulation, 0.59±0.21) compared with those with wild type gene (n=17) (○: before stimulation; 0.61±0.18, △: after IFN-β stimulation; 0.71±0.28). ($^{\#}P$=0.17). Relative expression level of *JAK1* gene induced by interferon-β (IFN-β) stimulation in patients with diabetes possessing heterozygous *TYK2* promoter variant (n=12) (●: before stimulation, ▲: after IFN-β stimulation) and homozygous patients (n=2) (●: before stimulation, ▲: after IFN-β stimulation) (before stimulation, 0.74±0.19; after stimulation, 0.82±0.17) compared with those with wild type gene (n=17) (○: before stimulation; 0.82±0.21, △: after IFN-β stimulation; 0.84±0.21) ($^{\#\#}P$=0.781). C. Relative expression level of ISGs induced by interferon-β (IFN-β) stimulation in patients with diabetes possessing heterozygous *TYK2* promoter variant (n=12) (●: before stimulation, ▲: after stimulation) and homozygous patients (n=2) (●: before stimulation, ▲: after stimulation) compared with those with wild type gene (n=17) (○: before stimulation, △: after stimulation). Results of relative expressions of ISGs are shown in patients with heterozygous and homozygous *TYK2* promoter variants. Before stimulation: *PKR*, 0.57±0.15; *OAS*, 0.58±0.20; *MxA*, 0.40±0.17. After stimulation: *PKR*, 0.79±0.27; *OAS*, 0.96±0.50; *MxA*, 0.77±0.54. In patients with wild type *TYK2* gene: before stimulation: *PKR*, 0.64±0.19; *OAS*, 0.62±0.33; *MxA*, 0.64±0.51. After stimulation: *PKR*, 0.79±0.18; *OAS*, 0.99±0.59; *MxA*, 1.02±0.75. The data are expressed as means ± standard deviations. ($^{\dagger}P$=0.94, $^{\dagger\dagger}P$=0.87, $^{\dagger\dagger\dagger}P$=0.30)

3. Supplementary Tables

Supplementary Table 1. Set of primers for amplification of *TYK2* gene.

	Forward	Reverse
Promoter Region	5'-GCCAGACCCCATCTCTACAAA-3'	5'-GGGAACACAAGCTCGAACC-3'
Exon 1	5'-AATCGCGGCTGAGTGACGAATG-3'	5'-GACCCAGACCCAGCTTTGAAGA-3'
Exon 2	5'-CTGGACATAAACTCTCCTAGGC-3'	5'-GACCATCTTGACCAACATGGTG-3'
Exon 3	5'-GTGGGTGGAAGGTTGAAGAG-3'	5'-GTGGATAGACGGATGGATGG-3'
Exon 4	5'-GGCTGACGGTAGCAAATGAC-3'	5'-CTGGGGCTTAGCACAGAGTC-3'
Exon 5	5'-GAAGCTGGTCTGACTCTGTGC-3'	5'-GCCCCTAAGTCTCCCACAA-3'
Exon 6	5'-CTCTGGGCTAGAGAGGAACG-3'	5'-GTCTACCCTGGCTCCCAGAT-3'
Exon 7	5'-ACCTGGCTAGTGTGCCTGTT-3'	5'-TCAGAGGCTAGGGTCAAGGA-3'
Exon 8	5'-GGAGGTATAAACGGGCATTG-3'	5'-GGAAATAGCCGTCCACCAG-3'
Exon 9	5'-GTAGGGGCTGGGCTAGGG-3'	5'-CCCCTAGGGCTCACAGTCTA-3'
Exon 10	5'-GGGTATGGGTCCAGAGTGG-3'	5'-GCAGAGGTGGGAGCAGTAAG-3'
Exon 11	5'-TACCGCCTGATCCTCACAGT-3'	5'-GCAGGCATCAAGTCATGGAG-3'
Exon 12	5'-GTGGGATGTGGCATCTCTCC-3'	5'-TGAAAGTTAGCAGCTGATCTCC-3'
Exon 13	5'-TGGGAGATCAGCTGCTAACTT-3'	5'-GCCACCTCCTCCACAGAC-3'
Exon 14	5'-GTGTGTCCGTGGAGGAGGT-3'	5'-GAGGGTTGGGGTACAGATCA-3'
Exon 15	5'-ATCCAGAGGGCAGAAGCAG-3'	5'-AGGCTGGTCTCGAACTCCTG-3'
Exon 16	5'-GTTGGCGTCTGTGCCTCT-3'	5'-GCGAAAGGAGCAGGGGAAG-3'
Exon 17	5'-CTTCCCCTGCTCCTTTCAC-3'	5'-AGAAGGGATGCAGCTTTGAG-3'
Exon 18	5'-GACTCCTCTGGGTCCCTTTC-3'	5'-CCTCTCGTGCGCTATAGGCA-3'
Exon 19	5'-TTTGTGACTCCCAAGTGTGG-3'	5'-CTCAACCCCCCAAACTCCTTC-3'
Exon 20	5'-CACCCACGCTCTAACCACGC-3'	5'-TGGTGCAGGGATTGGGGAGG-3'
Exon 21	5'-CTCTGCTGGGCTCAAGGTAG-3'	5'-CCCAAGCTGAAGAGGAAGG-3'
Exon 22	5'-CTCCTGGCTGCTCAGGTC-3'	5'-CTGGGATCATGCCCTATCAT-3'
Exon 23	5'-GATCCCCAAGCCCTCAGT-3'	5'-CCCAGCCTATGCCTTTCTAA-3'
Exon 24	5'-GCTGGGATTACAGGCATGAG-3'	5'-CCCTCTCCACAGCAGGATAG-3'
Exon 25	5'-CCTTTGTCTTTCCCTGACCC-3'	5'-CAGGGCTGCCATTGTGCCTC-3'

Supplementary Table 2. SNP at *TYK2* Exon 8 in patients with T1D, T2D and healthy controls.

SNP at Exon 8 (15597G/T)	Healthy Controls (n=254)	Type 1 DM				Type 2 DM (n=255)	
		All (n=244)		Flu-like syndrome* associated (n=36)			
	No (%)	No (%)	OR#(95% CI)	No (%)	OR#(95% CI)	No (%)	OR#(95% CI)
GG	115 (45.3%)	103 (42.2)	1.00¶	18 (50.0)	1.00¶	96 (37.6)	1.00¶
GT	116 (45.7%)	104 (42.6)	1.1 (0.8–1.6)	12(33.3)	0.8 (0.4–1.7)	121 (47.5)	1.3 (0.9–1.9)
TT	23 (9.0%)	37 (15.2)		6 (16.7)		38 (14.9)	
P value†		0.49		0.59		0.08	

*Symptoms of flu-like syndrome includes fever, chills, sore throat, muscle and joint aches, poor appetite, diarrhea, cough, and fatigue, suggestive of certain viral infections.

¶referent, #OR, odds ratio; §CI,confidence interval

†Heterozygous and homozygous variant genotypes combined versus homozygous wild genotype.

Supplementary Table 3. *TYK2* promoter variant in patients with T1D and with flu-like syndrome at the onset and of age from 20 to 39.

Genotype	TT1D (n=302)			T1D associated with flu-like syndrome (n=73)					T1D age 20-39 (n=107)					
				All[#]	age at onset (mea±SD)	Anti-GAD antibody[ʃ]			age at onset (mea±SD)	Anti-GAD antibody[ʃ]				with flu-like syndrome (n=23)
						Positive (≥1.5U/ml) (n=34)		Negative (<1.5U/ml) (n=39)		Positive (≥1.5U/ml) (n=69)		Negative (<1.5U/ml) (n=38)		
	No (%)	OR[#] (95% CI[§])	age at onset (mea±SD)	No (%)	OR (95% CI)	No (%)	OR (95% CI)	No (%) OR (95% CI)		No (%)	OR (95% CI)	No (%)	OR (95% CI)	No (%) OR (95% CI)
GT	273 (90.4)	1.00[¶]	28.0±18.1	63 (86.3)	1.00[¶]	25.3±17.2	31(91.4)	1.00[¶]	32(82.1) 1.00[¶]	28.2±5.6	63(91.3)	1.00[¶]	31(81.6)	1.00[¶] 19(82.3) 1.00[¶]
GT/AA	28 (9.3)	2.4 (1.2-4.6)	26.4±15.8	9 (12.3)	3.6 (1.5-8.5)	36.9±12.7	2(5.9)	2.2(0.6-8.0)	7(18.0) 5.0(1.9-13.2)	30.5±6.3	6(8.7)	2.1(0.8-5.8)	7(18.4)	5.1(1.9-13.6) 4(17.4) 4.8(1.4-15.9)
AA	1 (0.3)		25.6±16.2	1 (1.4)		33.5±16.0	1(2.9)		0(0.0)	30.5±6.3	0(0.0)		**0(0.0)**	0(0.0)
P-value	0.01[*]		0.48[‡]	0.005[*]		0.16[‡]	0.20[ʃ]		P=0.0005[ʃ]	P=0.17[‡]	P=0.12[ʃ]		P=0.0003[ʃ]	0.022[ʃ]

[*]Symptoms of flu-like syndrome include fever, chills, sore throat, muscle and joint aches, poor appetite, diarrhea, cough, and fatigue, suggestive of certain viral infections.

[¶]referent, [#]OR, odds ratio; [§]CI,confidence interval

[ʃ]Heterozygous (GT/AA) and homozygous (AA) variant genotypes combined (TYK2 promoter variant) versus homozygous wild genotype (GT) between the cases and healthy controls was statistically assessed by $\chi 2$ test. When the number of the patients of the group was less than 5, Fisher's exact test was used.

[‡]Statictical significance regarding age at onset between the wild and variant type were calculated by Student's t test.

[*]See Table 3.

T1D, type 1 diabetes.

Supplemental Table 4. *TYK2* promoter variant and obesity in patients with T2D.

Genotype	T2D (n=314)				
	ALL		BMI* (kg/m2)		
			ALL	≤26(n=257)	>26(n=57)
	No (%)	OR (95% CI)		No (%) OR (95% CI)	No (%) OR (95% CI)
GT	287 (91.4)	1.00¶	23.3±3.8	232(90.3) 1.00¶	55(96.5) 1.00¶
GT/AA	25 (8.0)	2.1 (1.1-4.1)	22.4±2.3	24(9.3) 2.4(1.2-4.8)	2(3.5) 0.8(0.2-3.7)
AA	2 (0.6)			1(0.4)	0(0.0)
P-value	0.03[ʃ]		0.12[‡]	0.01[ʃ]	1.0[ʃ]

*BMI: body mass index

[¶]referent, [#]OR, odds ratio; [§]CI,confidence interva

[ʃ]Heterozygous (GT/AA) and homozygous (AA) variant genotypes combined (TYK2 promoter variant) versus homozygous wild genotype (GT) between the cases and healthy controls was statistically assessed by $\chi 2$ test. When the number of the patients of the group was less than 5, Fisher's exact test was used.

[‡]Statictical significance regarding age at onset between the wild and variant type were calculated by Student's t test.